# A Reliable, Practical, and Economical Protocol for Inducing Diarrhea and Severe Dehydration in the Neonatal Calf

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## ABSTRACT

**Fifteen healthy, colostrum-fed, male dairy calves, aged 2 to 7 d were used in a study to develop a diarrhea protocol for neonatal calves that is reliable, practical, and economical. After instrumentation and recording baseline data, diarrhea and dehydration were induced by administering milk replacer [16.5 mL/kg of body weight (BW), PO], sucrose (2 g/kg in a 20% aqueous solution, PO), spironolactone and hydrochlorothiazide (1 mg/kg, PO) every 8 h, and furosemide (2 mg/kg, IM, q6h). Calves were administered sucrose and diuretic agents for 48 h to induce diarrhea and severe dehydration. Clinical changes after 48 h were severe watery diarrhea, severe depression, and marked dehydration (mean, 14% BW loss). Cardiac output, stroke volume, mean central venous pressure, plasma volume, thiocyanate space, blood pH and bicarbonate concentration, base excess, serum chloride concentration, and fetlock temperature were decreased. Plasma lactate concentration, hematocrit, and serum potassium, creatinine, phosphorus, total protein and albumin concentrations were increased. This noninfectious calf diarrhea protocol has a 100% response rate, while providing a consistent and predictable hypovolemic state with diarrhea that reflects most of the clinicopathologic changes observed in osmotic/maldigestive diarrhea caused by infection with rotavirus, coronavirus or cryptosporidia. Limitations of the protocol, when compared to infectious diarrhea models, include failure to induce a severe metabolic acidosis, absence of hyponatremia, renal instead of enteric loss of chloride, renal as well as enteric loss of free water, absence of profound clinical depression and suspected differences in the morphologic and functional effect on intestinal epithelium. Despite these differences, the sucrose/diuretic protocol should be useful in the initial screening of new treatment modalities for calf diarrhea. To confirm their efficacy, the most effective treatment methods should then be examined in calves with naturally-acquired diarrhea.**

## RÉSUMÉ

**Quinze veaux mâles en santé âgés de 2 à 7 jours et ayant reçu leur colostrum, ont été utilisés dans un protocole servant à simuler la diarrhée néonatale. La diarrhée et la déshydratation furent induites à l'aide du mélange suivant : lait de remplacement (16,5 mL/kg), sucrose (2 g/kg en solution aqueuse 20 %), spironolactone et hydrochlorothiazide (1 mg/kg) donné à toutes les huit heures, et du furosémide (2 mg/kg, im) administré à toutes les 6 heures. Les veaux ont reçu cette combinaison pendant 48 heures, à la fin desquelles une diarrhée sévère, une dépression marquée et une perte de poids (14 %) furent notées. Le volume d'éjection cardiaque, la pression veineuse centrale, le volume plasmatique, l'espace déterminé par le thiocyanate, le pH, les bicarbonates, les chlorures ainsi que la température des extrémités furent tous réduits. Par contre, l'hématocrite, les lactates, les ions potassium et phosphore, la créatinine, l'albumine et les protéines totales furent tous augmentés. Ce protocole produit des effets clinicopathologiques comparables à ceux d'une diarrhée associée à un syndrome de maldigestion avec augmentation de la pression osmotique telle que rencontrée lors de diarrhée aux virus rota, corona ou à la cryptosporidiose, dans 100 % des inductions réalisées. Ce modèle ne peut induire une acidose sévère, une hyponatrémie, une perte rénale de chlore au lieu d'intestinale, une perte d'eau rénale et intestinale, et provoque une dépression profonde et des différences possibles dans la morphologie et le fonctionnement de l'épithélium intestinal. Malgré ces différences, ce protocole peut être utile, dans un premier temps, pour vérifier l'efficacité de nouveaux traitements de la diarrhée des veaux avant de les appliquer à des veaux infectés.**

***(Traduit par docteur Pascal Dubreuil)***

## INTRODUCTION

Diarrhea in neonatal calves is a major source of economic loss to the cattle industry. Diarrhea is the leading cause of death in dairy heifer and beef calves aged less than 4 mo old, with annual mortality rates of 6.6% and 5.6% for dairy heifer and beef calves, respectively (1,2). Financial losses occur not only from calf mortality, but also from the cost of medication and labor needed to treat sick calves. Considering the economic importance of calf diarrhea, a consistent protocol for inducing diarrhea would facilitate comparison of treatment modalities and increase our understanding of the

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Received May 27, 1997.

pathophysiology of neonatal diarrhea. Development of a reliable, practical and economical protocol for inducing diarrhea in calves would provide an important research tool for the initial screening of new treatment modalities under controlled conditions.

Numerous methods have been used to experimentally induce diarrhea in neonatal calves. These methods can be grouped into 2 categories, infectious and non-infectious. The ideal model would be reliable, economical, and practical, while accurately reflecting the pathophysiologic changes associated with naturally-occurring diarrhea in calves. It would also minimize suffering and decrease the risk of pathogen transmission among calves, and decrease the risk of zoonotic disease in research workers. Infectious diarrhea models used in neonatal calves include enterotoxigenic *Escherichia coli* (3–9), *Cryptosporidium parvum* (10,11) coronavirus (12,13), rotavirus, or a combination of infectious agents (5,9,12,14–16). Non-infectious agents that have been administered orally to experimentally induce diarrhea in calves are sucrose (17–24), lactose (17,18,22,25–27), sorbitol (28), mannitol (29), castor oil (29), chloramphenicol, neomycin, ampicillin, and tetracycline (30,31), and purified $ST_a$ toxin from *E. coli* (32). Subcutaneous injection of reserpine has also been used (33).

Although each of these infectious and non-infectious models can cause diarrhea, most do not fulfill the criteria for an ideal calf diarrhea model. Problems include a large variation in response, ranging from no effect, to mortality despite treatment (3,6,8,9, 12,15,16), and an unpredictable time to the onset of diarrhea and dehydration (10–12,14–16). Such variability increases the number of calves needed to compare treatment modalities. The purpose of the study reported here was therefore to develop a reliable, economical, and practical diarrhea protocol that reflects the pathophysiology of naturally-occurring diarrhea. Sucrose was chosen to induce diarrhea because of predictable results in other studies (17–24) in inducing diarrhea. Diuretic agents were used in addition to sucrose to facilitate induction of dehydration.

## MATERIALS AND METHODS

### ANIMALS

The experimental protocol was approved by our institutional animal care and use committee. Fifteen healthy male dairy calves, colostrum-fed and aged between 2 and 6 d, were obtained from a local source. All calves had an adequate passive transfer of colostral immunoglobulins as evaluated by the serum total protein concentration; a reliable predictor of serum immunoglobulin concentration in normally hydrated calves (34). The calves were housed at an ambient temperature of 20 to 22°C in individual calf stalls and fed a high quality (all milk-based protein) milk replacer (Caf-gro Super Supreme, Farm & Fleet Stores Inc, Janesville, Wisconsin, USA) (crude protein > 22%, crude fat > 20%, crude fiber < 0.15%) at 10% of their BW/d, divided into 3 feedings at 7 AM, 3 PM, and 11 PM. The milk replacer was reconstituted according to manufacturers' directions at the rate of 227 g of dry powder per 1.892 mL of warm tap water (35–40°C).

### INSTRUMENTATION

Calves were acclimatized to their surroundings and diet until they were between 3 and 7 d of age. All calves had a normal appetite for the milk replacer and normal appearing and well formed feces before instrumentation. At this time, each calf was sedated with xylazine (Xylazine 20 mg/mL, Miles Inc, Animal Health Products, Shawnee Mission, Kansas, USA) (0.20 mg/kg, IM), and the right jugular furrow was clipped and surgically scrubbed for aseptic placement of an intravascular catheter. The catheter site was anesthetized with 1 mL of lidocaine hydrochloride (Lidocaine 2%, The Butler Company, Columbus, Ohio, USA) and an 8-F catheter introducer (Argon Medical, Edward Weck Inc, Athens, Texas, USA) placed using the Seldinger technique (35). A 90-cm 7F thermodilution catheter (Model 131H-7F Edwards, Swan-Ganz catheter, Baxter Healthcare Corporation, Edwards Critical Care Division, Irvine, California, USA) was advanced until the distal port was in the pulmonary artery and the proximal port was in or near the right atrium. The Swan-Ganz catheter was connected to calibrated fluid-filled transducers (P23XL transducer, Viggo Spectramed, Oxnard California, USA) and correct catheter position assessed by monitoring the pressure signals on a strip chart recorder (5/6 Recorder, Gilson Medical Electronics Inc, Middleton, Wisconsin, USA). The catheter introducer was then withdrawn from the jugular vein, and the Swan-Ganz catheter fastened to the neck. This Swan-Ganz catheter was used to determine cardiac output, mean central venous pressure, and blood temperature.

Fetlock temperature was measured by utilizing a thermistor on a second Swan-Ganz catheter. The thermistor was placed on the medial surface of the rear fetlock with a small amount of surgical lubricant (Johnson & Johnson Medical Inc, Arlington, Texas, USA) to facilitate heat conduction, and the entire Swan-Ganz catheter completely covered with tape (Johnson & Johnson Medical Inc) to insulate from room air. Xylazine sedation was reversed after instrumentation by administering tolazoline (1 mg/kg, IV) (Sigma Chemical Company, St. Louis, Missouri, USA) and the calf was placed in its movable stall. The proximal and distal ports of the intravascular Swan-Ganz catheter were flushed with heparinized saline (100 IU/mL) (Abbott Laboratories, North Chicago, Illinois, USA) every 8 h for the duration of the study.

A urine collection device was fitted to the calf after complete recovery from xylazine sedation. The urine collection device consisted of an empty 1 L intravenous fluid bag (Abbott Laboratories) with an IV drip set (Abbott Laboratories) attached and secured with tape (Johnson & Johnson Medical Inc). The opposite end of the bag was cut off and the bag placed around the prepuce of the calf and glued with a commercial adhesive (Goop, Eclectic Products, USA). The end of the IV drip set was placed through the bottom slats of the movable calf stall, and placed in a 1 L glass container to store the voided urine.

### BASELINE MEASUREMENTS

Following instrumentation, calves were kept in their movable stalls for at least 8 h before recording baseline values and obtaining baseline blood samples. During the period between

instrumentation and recording baseline values all calves had a normal appetite for milk replacer and normal appearing and well formed feces. Calves did not have access to water or feed.

Baseline values consisted of: heart rate; respiratory rate; rectal, fetlock and blood temperatures; cardiac output; mean central venous pressure; fecal consistency and pH; clinical hydration; depression status; urine volume; and BW. Blood samples were obtained from the distal port of the Swan-Ganz catheter for measurement of: mixed venous blood gas tension and blood pH; hematocrit; plasma lactate, glucose and protein concentration; serum biochemical analysis; plasma volume (Evans blue dye method); and extracellular volume (thiocyanate space).

Heart rate (beats per minute) was measured by auscultating the thorax with a stethoscope for a minimum of 30 s. Respiratory rate (breaths per minute) was measured by counting thoracic excursions for a minimum of 15 s; this was repeated if the calf was sniffing or vocalizing. Respiratory rate was obtained before any other measurements were taken and frequently with the calves apparently unaware of any human presence. Rectal temperature was measured (B-D thermometer, Becton Dickinson Consumer Products, Westminster, South Carolina, USA) before the calf was stimulated to defecate. A thermodilution cardiac output computer (COM-1 Cardiac output computer, American Edwards Laboratories) was used to measure blood temperature at the time of cardiac output determination, followed by fetlock temperature measurement utilizing the thermistor connector on the second Swan-Ganz catheter taped to the rear fetlock. Cardiac output was measured by injecting 5 mL of cold (0°C) 5% dextrose through the proximal port and monitoring the change in pulmonary artery temperature by a thermodilution cardiac output computer. The mean of 3 cardiac output determinations was used for the cardiac output at each time interval. Mean central venous pressure was determined by connecting a graduated pipette (Medex Water manometer set, American Pharmaseal Company, Medex Inc, Hilliard, Ohio, USA) filled with heparinized saline to the proximal port of the Swan-Ganz catheter, with the scapulohumeral joint taken as the zero pressure reference point. Measurements were taken with the calf facing forward, standing or in sternal recumbency, with the head in a normal alert, non-feeding position. Mean central venous pressure was defined as the height of the saline column (in cm $H_2O$) relative to the reference level.

Fecal consistency was determined and fecal samples for analysis were collected by digital rectal stimulation with a gloved hand. Fecal pH was measured immediately and the fecal sample retained for subsequent determination of fecal dry matter content. The amount of urine produced was measured in a graduated cylinder for each 8 h period, and urine production (in mL/h) at the end of the collection period calculated (collected urine volume/8 h).

Fecal consistency, the degree of clinical dehydration, and the degree of clinical depression were given numerical scores (24) as follows: *Fecal consistency*: 0 = normal, manure is normal and well formed; 1 = abnormal feces but not diarrhea, manure is pasty (softer than normal); 2 = mild diarrhea (semi-liquid, but still has a solid component); 3 = pure liquid feces. Diarrhea was defined as the voiding of feces that splashed when hitting the ground, with failure to form a firm cow pat. *Degree of clinical depression*: 0 = normal; 1 = mild depression, calf suckles but not vigorously; 2 = moderate depression, calf able to stand, suckling is weak or disorganized; 3 = severe depression, calf unable to stand or suckle. *Degree of clinical dehydration*: 0 = normal, eyes are bright and skin feels pliable; 1 = mild dehydration, slight loss of skin elasticity, skin tent < 3 s, eyes not recessed into orbit; 2 = moderate dehydration, skin tent > 3 s but < 10 s, eyes slightly recessed into orbit; 3 = severe dehydration, skin tent > 10 s, eyes markedly recessed into orbit. A total clinical score (TCS) was calculated by adding the 3 scores for the clinical evaluations described above, ranging in value from normal (0) to markedly abnormal (9).

Skin pliability was measured at the lateral thorax over the 6th to 9th rib, midway down the thorax by tenting and twisting the skin 90° for 1 s and then releasing the skin. Thoracic skin tent duration was defined as the time taken for the skin to return to its initial, non-tented, position. The extent of eyeball recession into the orbit was determined by gently rolling out the lower eyelid to its normal position and estimating the distance between the globe and palpebral conjunctiva. Hatch marks in 5 mm increments were made on the edge of the thumb used to pull down the eyelid to assist in estimating eyeball recession.

Body weight was measured by weighing the calf and the movable stall, and subtracting the weight of the stall. Calves were weighed after all measurements were taken and before administration of milk replacer; the BW therefore represented fasted BW. Each calf was stimulated to urinate and defecate by rubbing the perineal area before being weighed.

### EXPERIMENTAL PROTOCOL

Osmotic/maldigestive diarrhea and dehydration were induced by administering milk replacer solution (16.5 mL/kg, q8h; equivalent to a milk replacer feeding rate of 5% BW/d) and twice isotonic sucrose solution [2 g/kg sucrose (Flavorite Sugar, Chaska, Minnesota, USA) in a 20% aqueous solution; calculated 600 mOsm/L, PO, q8h], furosemide (Furosemide 50 mg/mL, Hoechst Roussel, Somerville, New Jersey, USA) (2 mg/kg, IM, q6h), spironolactone (Aldactazide, 50 mg tablets, G.D. Searle and Co, Chicago, Illinois, USA) (1 mg/kg, PO, q8h) and hydrochlorothiazide (Aldactazide, 50 mg tablets, G.D. Searle and Co) (1 mg/kg, PO, q8h). Calves suckled the milk replacer-sucrose solution readily. Calves were administered sucrose and diuretic agents for 48 h to induce diarrhea and severe dehydration. Calves were examined every 8 h at feeding time and the rectal temperature, heart rate, respiratory rate and urine volume recorded. At 24 and 48 h post-induction of diarrhea, mixed venous blood gas tension and blood pH, hematocrit, plasma protein concentration, cardiac output, blood temperature, fetlock temperature and BW were measured. Serum biochemical analysis, plasma lactate and glucose concentrations, fecal dry matter content, fecal pH, plasma volume, thiocyanate space, and hydration measurements

were determined at 48 h post-induction of diarrhea.

Plasma volume was determined by rapidly injecting 20 to 25 mg of Evans blue dye (Sigma Chemical Company), as a 0.5% solution, into the proximal port of the intravascular Swan-Ganz catheter, obtaining pulmonary artery blood from the distal port at 15, 20, 25, and 30 min after injection, and extrapolating the Evans blue dye concentration back to zero time as previously reported (36,37). Plasma volume determined by the Evans blue dye technique in calves is similar to that obtained using radiolabeled albumin (38). Blood volume was calculated from the plasma volume and hematocrit, after correcting for trapped plasma, whereby blood volume in L = [plasma volume (L) × 100]/[100 − (hematocrit % × 0.94)] (39).

Thiocyanate space (correlated with extracellular fluid volume) (40) was determined by slowly injecting 1.0 to 1.1 g of sodium thiocyanate (Sigma Chemical Company), made up as a 3.3% solution, into the proximal port of the intravascular Swan-Ganz catheter over 1 min. Pulmonary artery blood samples were obtained from the distal port of the intravascular Swan-Ganz catheter at 60, 80, 100 and 120 min after injection. Thiocyanate space was determined by extrapolating the thiocyanate concentration back to zero time as previously reported (36). Thiocyanate space in calves is similar to that obtained by radiolabeled sodium (38).

Forty-eight hours after induction of watery diarrhea and severe dehydration, the 15 calves were randomly assigned to 3 groups (2 treatment and 1 control), and the results are reported elsewhere (41). All 15 calves (including 5 control calves) survived the 24 h treatment phase, and at the end of the 24 h treatment phase, diarrhea stopped within 8 h of cessation of sucrose administration.

### SAMPLE ANALYSIS

Automated methods were used to measure serum sodium, potassium, and chloride concentrations (Ion-Selective electrode), serum phosphate concentration (ammonium phosphomolybdate), serum creatinine concentration (Jaffe picric acid), serum albumin concentration (bromcresol green), and serum total protein concentration (biuret) (Hitachi 704 Automatic Analyzer, Tokyo, Japan). Plasma glucose (glucose oxidase) and lactate (oxidase) concentrations were also determined (YSI model 2300 Stat Plus Glucose and L-Lactate Analyzer, Yellow Springs, Ohio, USA). Blood for serum and plasma analysis was kept at 4°C for 2–4 h before being centrifuged, the serum and plasma was then separated and frozen at −20°C for less than 2 mo before being analyzed. Hematocrit was determined by microcentrifugation and plasma and serum protein concentration by refractometry. Samples for blood pH, blood gas tension, hemoglobin concentration, and plasma ionized calcium concentration were analyzed by a blood gas analyzer (238 pH/blood gas analyzer, Ciba Corning, Halstead, England) and the blood pH and blood gas tension values corrected for rectal temperature. Samples for blood pH and blood gas tension analysis were kept at 4°C before being analyzed within 4 h.

Evans blue dye concentration in plasma was determined spectrophotometrically (Coleman 55 Spectrophotometer, Coleman Instruments Division, Oak Brook, Illinois, USA) at 620 nm by interpolation from a standard curve. To prepare a standard curve for each calf, 1 mL of pre-injection plasma was added to 1 mL of a known concentration of Evans Blue Dye in isotonic saline and the absorbance measured against a plasma blank. Preparation of the samples consisted of taking 1-mL aliquots each of the post-injection plasma and isotonic saline and measuring absorbance at 620 nm against the pre-injection plasma blank. All plasma volume samples were analyzed within 24 h of collection, after storage at 4°C.

Plasma thiocyanate concentration was determined spectrophotometrically (Coleman 55 Spectrophotometer, Coleman Instruments Division, Oak Brook, Illinois, USA) after deproteinization of plasma to remove Evans Blue Dye. This process involved adding 1 mL of plasma to 10 mL of cold 10% trichloroacetic acid (TCA) (EK Industries, Addison, Illinois, USA), centrifuging for 10 min and removing the supernatant. The supernatant was stored at room temperature for up to 3 wk. The supernatant was mixed 1:4 with 16% ferric nitrate (Mallinckrodt Inc., Paris, Kentucky, USA) solution, covered with a dark cloth for 15 min, and the absorbance immediately read at 460 nm against a reagent blank (2 mL of TCA and 1.5 mL ferric nitrate reagent) (42). The pre-injection plasma absorbance was subtracted from the thiocyanate-containing plasma samples and the concentration of sodium thiocyanate determined from the standard curve as previously described (36).

A Jenco portable pH meter (Jenco Model 6009, Micro Computer based portable pH meter, San Diego, California, USA) was used to measure fecal pH levels (immediately after collection) by total immersion of the probe in the feces; the average of 2 such measurements was taken as the fecal pH value. Fecal dry matter content was determined by placing a known amount of feces in a ceramic crucible and drying at 60°C. Each sample was weighed every 3 d until the weight remained constant; the fecal dry matter content was then calculated as: [(fecal dry weight)/(fecal wet weight)] × 100.

### STATISTICAL ANALYSIS

Data are presented as means ± $s_{\bar{x}}$. Paired *t*-tests were used to compare normally distributed continuous variables and Wilcoxon signed rank tests were used to compare ordinal and non-normally distributed variables. The normalcy of the distribution was evaluated by examining the skewness and kurtosis of the data and calculating the Shapiro-Wilk statistic. Variables with non-normal distributions or unequal variances were log transformed before being compared by a paired *t*-tests. Paired comparisons were between baseline and time = 48 h values only. The time = 24 h values were not statistically compared to the other values, but were included for descriptive purposes. A $P$ value $< 0.05$ was considered significant.

## RESULTS

The administration of sucrose and 3 different diuretic agents resulted in profuse watery diarrhea, moderate dehydration and an average weight loss of 3.9 kg, equivalent to 10% of

initial BW, in 24 h without evidence of profound depression. Severe watery diarrhea (fecal score 3) was evident in all calves within 8 h of starting sucrose administration, and was maintained the entire time that sucrose was fed. This clinical observation was consistent with the significant decrease in fecal dry matter content (Table I). Fecal pH decreased significantly from 5.89 to 5.10 following diarrhea induction with sucrose. Watery diarrhea was maintained until 48 h, at which time the average BW loss was 5.4 kg, corresponding to 14% dehydration (Table I). Calves had clinical evidence of severe dehydration at that time, as measured by eyeball recession into orbit (7.7 mm), prolonged thoracic skin tent duration (> 6 s for all calves), increased hydration score, rectal-fetlock temperature difference, and decreased urine production (Table I). All calves were examined every 8 h and monitored periodically between examination/treatment periods. Calves preferred to stay in sternal recumbency between examination periods, but would stand and suckle at feeding time. If at any time throughout the study a calf received a clinical depression score of 3 (unable to stand or suckle) the calf was immediately euthanized. All calves survived the 48 h diarrhea/dehydration induction phase (including all 5 calves in the control group), and were able to stand and suckle throughout this period.

Rectal and blood temperatures were significantly increased by 48 h, whereas fetlock temperature decreased by 6.4°C in 24 h and by 7.8°C in 48 h (Table I). The rectal-fetlock temperature difference also increased from 4.8°C to 13.1°C by 48 h. The large reduction in fetlock temperature was consistent with the clinical observation that all extremities were cool to the touch. Heart rate and respiratory rate were not significantly changed by 48 h; however, it appeared that depth of respiration increased toward the end of the 48 h time period.

Plasma volume decreased by 26% and thiocyanate space decreased by 33% after 48 h of diarrhea and dehydration (Table I). The reduction in plasma volume and thiocyanate space was accompanied by a significant decrease in cardiac output, stroke volume, mean central venous pressure, and blood volume (Table I). The reduction in thiocyanate space (5.8 L) was greater than the reduction in BW (5.4 kg), indicating that fluid and weight loss was primarily from the extracellular space, and that there may have been some expansion of the intracellular space.

Hypovolemia and decreased glomerular filtration rate were reflected by significant increases in hematocrit, hemoglobin concentration, plasma protein concentration, and serum concentrations of albumin, total protein, creatinine, and phosphate (Table II). Serum sodium concentration was unchanged, whereas serum potassium concentration increased and serum chloride concentration decreased. Hyperphosphatemia was accompanied

**Table I. Physical examination findings, hemodynamic values, and fecal characteristics in neonatal dairy calves ($n$ = 15) with experimentally-induced diarrhea**

| | Baseline | 24 h | 48 h | Probability |
|---|---|---|---|---|
| Physical examination findings | | | | |
| Body weight (kg) | 39.6 ± 2.6 | 35.7 ± 2.3 | 34.2 ± 2.3 | < 0.0001 |
| Rectal temperature (°C) | 39.1 ± 0.1 | 39.6 ± 0.1 | 39.6 ± 0.1 | 0.0082 |
| Blood temperature (°C) | 39.2 ± 0.1 | 39.6 ± 0.1 | 39.6 ± 0.1 | 0.011 |
| Fetlock temperature (°C) | 34.3 ± 0.7 | 27.9 ± 1.2 | 26.5 ± 0.9 | < 0.0001 |
| Rectal-fetlock temperature difference | 4.8 ± 0.7 | 11.7 ± 1.1 | 13.1 ± 3.7 | 0.0001 |
| Heart rate (beats/min) | 126 ± 4 | 137 ± 4 | 131 ± 7 | NS |
| Respiratory rate (breaths/min) | 38 ± 2 | 35 ± 2 | 37 ± 2 | NS |
| Thoracic skin tent duration (s) | 2.1 ± 0.1 | ND | 8.9 ± 1.7 | < 0.0001 |
| Eyeball recession into orbit (mm) | 0 ± 0 | ND | 7.7 ± 0.4 | < 0.0001 |
| Hydration score (0 to 3) | 0 ± 0 | 1.9 ± 0.2 | 2.9 ± 0.1 | < 0.0001 |
| Clinical depression score (0 to 3) | 0 ± 0 | 0.3 ± 0.1 | 1.2 ± 0.2 | < 0.0001 |
| Total clinical score (0 to 9) | 0 ± 0 | 5.2 ± 0.2 | 7.1 ± 0.2 | < 0.0001 |
| Hemodynamic values | | | | |
| Thiocyanate space (L) | 17.4 ± 1.3 | ND | 11.6 ± 0.8 | < 0.0001 |
| Plasma volume (L) | 3.4 ± 0.3 | ND | 2.5 ± 0.2 | < 0.0001 |
| Estimated interstitial fluid volume (L) | 14.0 ± 1.3 | ND | 9.1 ± 0.7 | < 0.0001 |
| Blood volume (L) | 4.6 ± 0.4 | ND | 3.9 ± 0.3 | 0.0130 |
| Plasma volume/Thiocyanate space (%) | 20.6 ± 1.6 | ND | 22.2 ± 1.5 | NS |
| Cardiac output (L/min) | 8.80 ± 0.42 | 4.47 ± 0.26 | 3.89 ± 0.26 | < 0.0001 |
| Stroke volume (mL) | 71 ± 4 | 33 ± 2 | 32 ± 3 | < 0.0001 |
| Mean central venous pressure (cm $H_2O$) | 0.6 ± 0.8 | ND | −1.9 ± 1.0 | 0.0029 |
| Urine production (mL/h) | 81 ± 15 | 61 ± 10 | 12 ± 6 | < 0.0001 |
| Fecal characteristics | | | | |
| Fecal consistency score (0 to 3) | 0 ± 0 | 3.0 ± 0 | 3.0 ± 0 | < 0.0001 |
| Fecal dry matter content (%) | 28 ± 2 | ND | 9 ± 1 | < 0.0001 |
| Fecal pH | 5.89 ± 0.10 | ND | 5.10 ± 0.09 | < 0.0001 |

Values are mean ± $s_{\bar{x}}$
ND = not determined
NS = not significant

**Table II. Laboratory findings in neonatal dairy calves ($n$ = 15) with experimentally-induced diarrhea**

| | Baseline | 24 h | 48 h | Probability |
|---|---|---|---|---|
| Mixed venous pH | 7.36 ± 0.01 | 7.35 ± 0.01 | 7.31 ± 0.02 | 0.0029 |
| Mixed venous $P_{CO2}$ (mmHg) | 60 ± 1 | 64 ± 1 | 60 ± 2 | NS |
| Mixed venous $P_{O2}$ (mmHg) | 31 ± 1 | 27 ± 1 | 32 ± 1 | NS |
| Mixed venous $HCO_3^-$ (mEq/L) | 34.3 ± 0.5 | 35.9 ± 1.1 | 31.0 ± 1.6 | 0.0172 |
| Mixed venous Base excess (mEq/L) | +8.6 ± 0.6 | +9.9 ± 1.2 | +4.7 ± 1.8 | 0.015 |
| Plasma lactate (mM/L) | 1.1 ± 0.1 | ND | 2.8 ± 0.5 | 0.0008 |
| Hemoglobin (g/dL) | 7.5 ± 0.2 | 9.6 ± 0.3 | 9.8 ± 0.3 | < 0.0001 |
| Hematocrit (%) | 28.2 ± 1.2 | 32.5 ± 1.0 | 36.9 ± 1.3 | < 0.0001 |
| Plasma protein (g/dL) | 6.4 ± 0.2 | 7.5 ± 0.3 | 8.1 ± 0.2 | < 0.0001 |
| Serum sodium (mEq/L) | 136 ± 1 | ND | 139 ± 2 | NS |
| Serum potassium (mEq/L) | 4.8 ± 0.1 | ND | 6.4 ± 0.5 | 0.017 |
| Serum chloride (mEq/L) | 94 ± 1 | ND | 90 ± 2 | 0.033 |
| Plasma calcium (mEq/L) | 5.2 ± 0.3 | ND | 4.5 ± 0.1 | < 0.0001 |
| Serum phosphorus (mg/dL) | 7.0 ± 0.3 | ND | 11.0 ± 0.3 | < 0.0001 |
| Serum total protein (g/dL) | 5.6 ± 0.2 | ND | 7.4 ± 0.4 | 0.0005 |
| Serum albumin (g/dL) | 2.6 ± 0.1 | ND | 3.4 ± 0.1 | < 0.0001 |
| Serum creatinine (mg/dL) | 0.8 ± 0.1 | ND | 5.1 ± 0.6 | < 0.0001 |
| Plasma glucose (mg/dL) | 68 ± 4 | ND | 66 ± 5 | NS |

Values are mean ± $s_{\bar{x}}$
ND = not determined
NS = not significant

by a decrease in plasma ionized calcium concentration.

Despite the severe dehydration induced by sucrose and diuretic administration, calves developed only a mild metabolic acidosis, as indicated by a small decrease in blood pH, bicarbonate concentration, and base excess, and a corresponding small increase in plasma lactate concentration (Table II).

## DISCUSSION

The oral administration of sucrose, spironolactone, and hydrochlorothiazide, and IM administration of furosemide, provided a reliable, practical, and economical protocol for inducing diarrhea and severe dehydration in neonatal calves. Watery diarrhea was induced in all calves within 8 h, and maintained for the entire time that sucrose was administered. A predictable and severe dehydrated state was also induced in all calves, with the severity of the dehydration increasing with time.

Calves do not possess intestinal sucrase and are therefore unable to utilize dietary sucrose (18,43). This makes sucrose an unsuitable energy source for feeding calves, but an ideal agent for inducing diarrhea. As such, sucrose has been used in numerous studies to reliably and rapidly induce watery diarrhea in neonatal calves (17–24). Sucrose-induced diarrhea is associated with decreased intestinal transit time and increased fecal bacterial concentration (17,18,23); both changes are identical to those observed in calves with naturally-acquired diarrhea (25,44). Sucrose is fermented in the small intestine by enteric bacteria, with metabolism of 38% and 41% of an ingested sucrose load in the small intestine of 4–6 mo old steers and 4 wk old calves, respectively (45,46). Sucrose fermentation to volatile fatty acids also occurs in the large intestine, thereby lowering fecal pH (46), as observed in the present study. Sucrose can therefore be regarded as a classical osmotic/maldigestive diarrhea agent in calves. Interestingly, the change in fecal pH induced by feeding sucrose (decrease from 5.9 to 5.1) was similar in magnitude and direction to that observed in naturally-acquired undifferentiated diarrhea in calves (decrease in fecal pH from 6.8 to 6.0) (25). In contrast, fecal pH increased from 5.5 to 7.1 in calves with experimentally-induced enterotoxigenic *E. coli* diarrhea, a classical secretory diarrhea agent (47).

The mean dry matter content of normal calf feces is 24% (48), whereas the dry matter content of calf feces classified as loose or watery was 13%, and 9%, respectively (25). In the study reported here, we observed a decrease in fecal dry matter content from 28% to 9%, consistent with sucrose-induced diarrhea. Reduction in fecal dry matter content induced by sucrose is thought to facilitate bacterial multiplication in the large intestine and increase bacterial concentrations in large intestinal fluid and feces (23), potentially exacerbating electrolyte and acid base derangements associated with diarrhea.

Whitten et al conducted a study to measure intestinal exchanges of electrolytes in healthy calves and calves with diarrhea. Two calves contracted diarrhea spontaneously and 2 calves had sucrose-induced diarrhea when fed as an isotonic formulation (2 g sucrose/kg, 10% aqueous solution, q8h). Histologic changes in the intestine were similar in calves with naturally-acquired and sucrose-induced diarrhea, and included shortening and blunting of the intestinal villi and damage to the lamina propria (21). In another study to determine the effect of sucrose administration on susceptibility to *Cryptosporidium parvum* infection, infant mice were administered oral hypertonic sucrose solution to evaluate the interaction of an intestinal lectin-like receptor with carbohydrates. Histologic examination showed marked vacuolation of jejunal and ileal epithelium after 1 d of hypertonic sucrose solution administration with return to normal tissue 3 d after cessation of treatment (49). Although unsubstantiated; similar histologic lesions may have been present in the calves in this study, as we fed sucrose in a hypertonic formulation (2 g sucrose/kg, 20% aqueous solution, q8h) and hypertonic solutions can directly damage intestinal epithelium, particularly at the villus tip (50).

Sucrose administration (without concurrent administration of diuretic agents) induces fecal losses of water, sodium, and potassium similar to those in calves with naturally-acquired diarrhea (19,20), but results in a smaller fecal chloride loss (19,20). Accordingly, 3 diuretic agents with different sites of action were used in the present study to produce electrolyte losses in calves administered sucrose similar to those occurring in calves with naturally-acquired diarrhea. Furosemide is a loop diuretic which inhibits the reabsorption of sodium in the proximal and distal tubules and in the ascending limb of the loop of Henle. Furosemide is a potent diuretic, natriuretic, and chloruretic agent in the presence of acidosis or alkalosis, and has no inhibitory effect on carbonic anhydrase or aldosterone activity in the distal tubule (51,52). Furosemide is widely used in cattle to induce diuresis and treat udder edema (53,54), but tends to induce hypokalemia in most species (52,54). Hydrochlorothiazide promotes the excretion of sodium and water primarily by inhibiting their reabsorption in the cortical diluting segment of the distal renal tubule. Its principal action is enhancement of the excretion of approximately equal amounts of sodium and chloride (51,55,56). Hypokalemia may develop as a result of profound diuresis, particularly when used concomitantly with loop diuretics such as furosemide (51,55). Like furosemide, hydrochlorothiazide is used in cattle to induce diuresis and treat udder edema (53,56). Spironolactone acts as a specific pharmacologic aldosterone antagonist, primarily by competitive binding of receptors at the aldosterone-dependent sodium-potassium exchange site in the distal convoluted renal tubule (51). Spironolactone will therefore antagonize the hypokalemic effect of furosemide and hydrochlorothiazide, but exert a weaker diuretic, natriuretic, and chloruretic effect than furosemide (51). In 4-week-old calves, spironolactone (1 mg/kg, PO, q24h) increased the renal excretion of sodium and chloride and inhibited aldosterone activity, resulting in decreased plasma volume, decreased plasma sodium and chloride concentrations, and increased plasma potassium concentration (57).

The severe dehydration (14% BW) produced in this study was not accompanied by mortality or severe

acidemia. All calves were able to stand and suckle at feeding time with this degree of dehydration but preferred to stay in sternal recumbency between examination periods. Previous calf studies have indicated that calves with diarrhea die when they have lost 12.7–13.4% of their BW (19,58), and guidelines have been developed that suggest a 12–14% decrease in BW due to dehydration is fatal (59,60). The results of the study reported here clearly suggest that death from diarrhea is not solely due to dehydration, but more likely due to acid-base and electrolyte derangements, specifically severe acidemia and hyperkalemia (19,61–66). Results of previous calf diarrhea studies indicate that the primary cause for acidemia is excessive loss of bicarbonate in the diarrheic feces (15,19, 21,61,65), rather than severe dehydration and accompanying lactic acidosis. Blood or plasma lactate concentrations do not usually increase above 3 mM/L in calves with diarrhea, but can rapidly increase above 3 mM/L during the final hours before death (67,68). Lactate concentration can vary with the age of the calf, with calves less than 1 wk old having a greater tendency to develop lactic acidosis (70).

Dehydration in calves with diarrhea is accompanied by a large decrease in extracellular fluid volume and a smaller increase in intracellular fluid volume (14). During diarrhea there is increased intestinal loss of sodium, potassium, and chloride, with a concurrent decrease in plasma sodium concentration, resulting in hypo-osmotic plasma and extracellular fluid (14,20,69–72). The diarrhea-induced extracellular hypo-osmolality causes free water to move from the extracellular to intracellular fluid space, thereby increasing the latter. Intracellular fluid volume may also increase independently of extracellular hypo-osmolality changes in severely dehydrated diarrheic calves, as hypovolemic shock results in cellular hypoxia, partial depolarization of the resting membrane potential, and increased intracellular sodium concentration, resulting in cellular swelling (61,73). Whatever the mechanism, the increase in intracellular fluid volume in calves with diarrhea is at the expense of extracellular fluid and therefore contributes to the development of hypovolemic shock in affected calves. In the study reported here, the decrease in thiocyanate space, which is correlated with extracellular volume (36,38), was slightly greater than the decrease in BW, 5.8 L and 5.4 kg respectively, indicating that fluid and weight loss in this calf diarrhea model was primarily from the extracellular space, and that there may have been some expansion of the intracellular space. The ratio of plasma volume to thiocyanate space remained constant before (0.21) and after (0.22) diarrhea induction, indicating that water was not lost preferentially from the intravascular or interstitial compartments. Taken together, these findings indicate that the sucrose/diuretic calf diarrhea protocol produced similar alterations in body fluid compartments as seen in experimentally-induced viral diarrhea (14) and naturally-acquired diarrhea (69). However, because diuretic agents were administrated to facilitate extracellular fluid loss, the route of free water loss in the sucrose/diuretic protocol (intestinal and renal) differs from that seen in naturally-acquired diarrhea (intestinal only).

Measurement of fetlock temperature may be clinically valuable in calves with diarrhea, as an indicator of peripheral blood flow and response to treatment. Most calves with severe dehydration and diarrhea have cold extremities due to poor peripheral perfusion. Phillips and Lewis and Jones et al measured fetlock (dorsal aspect) and hock (dorsal aspect) temperatures in calves with experimentally-induced or naturally-acquired diarrhea (14,74), and found that fetlock temperature appeared to be more sensitive to changes in hydration status. Normal fetlock temperature was 34 to 37°C, and decreased to 26°C after the onset of diarrhea (14). The changes in fetlock temperature observed in the study reported here (decrease from 34 to 27°C over 48 h) closely followed those in calves with experimentally-induced and naturally-acquired viral diarrhea (14,74). Because diarrheic calves may be hypothermic or pyrexic, the difference between rectal temperature and fetlock temperature is likely to provide a more accurate guide to peripheral blood flow than the fetlock temperature alone. A rectal-fetlock temperature difference of 13°C was reported in one comatose and near death calf with experimentally-induced coronavirus diarrhea (67). In the study reported here, we found the normal rectal-fetlock temperature difference to be 4–5°C in calves kept at an ambient temperature of 20–22°C, and the temperature difference to increase to 13°C in severely dehydrated calves. Other studies have reported that the normal rectal-fetlock temperature difference in neonatal calves was 2–3°C, and that the temperature difference increased to 8–15°C in calves with experimentally-induced dehydration (29–35% reduction in plasma volume) (7,75). Further evaluation of the rectal-fetlock temperature difference in dehydrated calves with diarrhea is indicated in order to determine its clinical utility.

The protocol reported here, combining sucrose and diuretic agents, can be classified as an osmotic/maldigestive diarrhea, and is therefore functionally similar to diarrhea caused by rotavirus, coronavirus, and cryptosporidia (28). A similar protocol using only one diuretic agent (furosemide) and a shorter duration of treatment (24 h) produced severe watery diarrhea and moderate dehydration (24), but was not used for the present study because pilot studies in 8 calves indicated that it was incapable of producing severe dehydration (> 10% decrease in BW), within 48 h. Biochemical changes commonly observed in calf diarrhea include prerenal azotemia, metabolic acidosis, mild hyponatremia and hyperlactatemia, a variable degree of hyperkalemia and hyperphosphatemia, and hypoglycemia (13,76,77). Serum chloride concentration usually is unchanged (58). The sucrose/diuretic diarrhea protocol used in this study produced marked prerenal azotemia and hyperkalemia, mild metabolic acidosis, hyperphosphatemia, hypocalcemia, hypochloremia, and hyperlactatemia, with no change in serum sodium concentration. This suggests that the protocol causes slightly different electrolyte losses than that associated with naturally-acquired diarrhea.

The sucrose/diuretic protocol was economical, with an average cost of

$9 for a 50 kg calf, excluding the cost of housing and feed. The protocol had a 100% response rate within 12 h with no mortality within 48 h, in contrast to *E. coli* diarrhea models that had a 53% to 100% response rate in 1 to 4 d, with up to 85% mortality (3,5,7,8). A similar trend is seen in models that use coronavirus/rotavirus, with a 50% to 100% response rate in 1 to 6 d, and 33 to 86% mortality (12,14–16,61, 71,73), and *Cryptosporidium*, with a 86 to 100% response rate in 3 to 6 d (10,11).

Initial studies using sucrose investigated the capacity of the neonatal calf to utilize various carbohydrates as an energy source in milk replacer formulations. To that end it was discovered that neonatal calves were unable to hydrolyze sucrose and therefore unable to utilize sucrose as an energy source (17,22). In addition it was noted that diarrhea resulted within several h of feeding sucrose, but feces always returned to normal consistency within 8–12 h of withdrawing sucrose from the diet (18). Additional research followed utilizing sucrose as a method to create diarrhea in fluid compartmentalization studies when calves did not develop diarrhea spontaneously (19,20,21). Although administration of sucrose (isotonic solution) to calves will be 100% effective in creating diarrhea, calves will only become mildly (< 6%) dehydrated in 48 h. The addition of furosemide (2 mg/kg, IM, q6h) to the sucrose protocol produced electrolyte losses in calves similar to naturally-acquired diarrhea and induced moderate dehydration (8%) but was incapable of producing severe dehydration (24). In the protocol reported here, 2 additional diuretic agents with different mechanisms of action (hydrochlorothiazide and spironolactone) were also administered to hasten the development of severe dehydration and more closely mimic the electrolyte imbalances induced by naturally-acquired diarrhea.

In summary, although the sucrose/diuretic protocol reliably induced diarrhea and severe dehydration in neonatal calves, it has some limitations. As stated previously, these limitations include failure to induce a severe metabolic acidosis (possibly because of decreased intestinal loss of bicarbonate compared to naturally-acquired diarrhea), a different route for chloride loss (renal instead of enteric), 2 routes for free water loss (enteric and renal) instead of one route in naturally-acquired diarrhea, failure to induce hyponatremia, lack of profound clinical depression, and possible differences in the morphologic and functional alterations in intestinal epithelium, when compared to viral or *E. coli* diarrhea. We therefore suggest that the sucrose/diuretic protocol is useful for the initial screening of fluid resuscitation methods in treating moderately to severely dehydrated calves with diarrhea that are not severely acidemic. To confirm their efficacy, the most effective treatment methods should then be examined in calves with naturally-acquired diarrhea.

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